CLAIMS

We claim,

1. A method of replacing, in whole or in part, in a non-human eukaryotic cell, an endogenous immunoglobulin variable region gene locus with an homologous or orthologous human gene locus comprising:
   a) obtaining a large cloned genomic fragment containing, in whole or in part, the homologous or orthologous human gene locus;
   b) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (a) to create a large targeting vector for use in the eukaryotic cells (LTVEC);
   c) introducing the LTVEC of (b) into the eukaryotic cells to replace, in whole or in part, the endogenous immunoglobulin variable gene locus; and
   d) using a quantitative assay to detect modification of allele (MOA) in the eukaryotic cells of © to identify those eukaryotic cells in which the endogenous immunoglobulin variable region gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus.

2. The method of claim 1 further comprising:
   e) obtaining a large cloned genomic fragment containing a part of the homologous or orthologous human gene locus that differs from the fragment of (a);
   f) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (e) to create a second LTVEC;
   g) introducing the second LTVEC of (f) into the eukaryotic cells identified in step (d) to replace, in whole or in part, the endogenous immunoglobulin variable gene locus; and
   h) using a quantitative assay to detect modification of allele (MOA) in the eukaryotic cells of (g) to identify those eukaryotic cells in which the endogenous immunoglobulin variable region gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus.

3. The method of claim 2 wherein steps (e) through (h) are repeated until the endogenous immunoglobulin variable region gene locus is replaced in whole with an homologous or orthologous human gene locus.

4. The method of claim 1 wherein the immunoglobulin variable gene locus is a locus selected from the group consisting of :
   a) a variable gene locus of the kappa light chain;
   b) a variable gene locus of the lambda light chain; and
   c) a variable gene locus of the heavy chain.

5. The method of claim 4 wherein the quantitative assay comprises quantitative PCR, FISH, comparative genomic hybridization, isothermic DNA amplification, or quantitative hybridization to an immobilized probe.

6. The method of claim 5 wherein the quantitative PCR comprises TaqMan® technology or quantitative PCR using molecular beacons.

7. A method of replacing, in whole or in part, in a mouse embryonic stem cell, an endogenous immunoglobulin variable region gene locus with its homologous or orthologous human gene locus comprising:
   a) obtaining a large cloned genomic fragment containing, in whole or in part, the homologous or orthologous human gene locus;
   b) using bacterial homologous recombination to genetically modify the large cloned genomic fragment of (a) to create a large targeting vector for use in the embryonic stem cells;
   c) introducing the large targeting vector of (b) into embryonic stem cells to replace, in whole or in part, the endogenous immunoglobulin variable gene locus in the cells; and
   d) using a quantitative PCR assay to detect modification of allele (MOA) in the embryonic stem cells of © to identify those embryonic stem cells in which the endogenous variable gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus.

8. The method of claim 7 further comprising:
   e) obtaining a large cloned genomic fragment containing a part of the homologous or orthologous human gene locus that differs from the fragment of (a);
   f) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (e) to create a large targeting vector for use in the embryonic stem cells;
   g) introducing the large targeting vector of (f) into the embryonic stem cells identified in step (d) to replace, in whole or in part, the endogenous immunoglobulin variable gene locus; and
   h) using a quantitative assay to detect modification of allele (MOA) in the mouse embryonic stem cells of (g) to identify those embryonic stem cells in which the endogenous immunoglobulin variable region gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus.

9. The method of claim 8 wherein steps (e) through (h) are repeated until the endogenous immunoglobulin variable region gene locus is replaced in whole with an homologous or orthologous human gene locus.

10. The method of claim 7 wherein the immunoglobulin variable gene locus comprises a locus selected from the group consisting of
   a) a variable gene locus of the kappa light chain;
   b) a variable gene locus of the lambda light chain; and
   c) a variable gene locus of the heavy chain.

11. A method of replacing, in whole or in part, an endogenous immunoglobulin variable region gene locus with an homologous or orthologous gene locus comprising:

   a) creating a LTVEC comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin

variable gene locus region and an upstream homology arm within the variable gene locus;

b) creating a LTVEC comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region and a downstream homology arm within the variable gene locus;

c) introducing the LTVEC s of (a) and (b) into the eukaryotic cell;

d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify those eukaryotic cells in © in which the site-specific recombination sites flank the endogenous variable region gene locus;

e) creating a vector containing the site-specific recombination sequences flanking all or part of the orthologous or homologous gene locus; and

f) introducing the vector of (e) into the eukaryotic cells identified in step (d) such that, through recombination, the endogenous immunoglobulin variable region gene locus is replaced, in whole or in part, with the homologous or orthologous gene locus.

12. The method of claim 11 wherein the eukaryotic cell is an embryonic stem cell.

13. A genetically modified immunoglobulin variable region gene locus produced by the method of claim 1, 4, 7, 8, 10, 11 or 12.

14. A genetically modified eukaryotic cell comprising a genetically modified immunoglobulin variable region gene locus produced by the method of claim 1, 4, 7, 8, 11 or 12.

15. A non-human organism comprising a genetically modified immunoglobulin variable region gene locus produced by the method of claim 1, 4, 7, 8, 10 or 11.

16. A mouse embryonic stem cell containing a genetically modified immunoglobulin variable region gene locus produced by the method of claim 7, 8, 10 or 12.

17. An embryonic stem cell of claim 16 wherein the mouse heavy chain variable region locus is replaced, in whole or in part, with a human heavy chain variable gene locus.

18. An embryonic stem cell of claim 16 wherein the mouse kappa light chain variable region locus is replaced, in whole or in part, with a human kappa light chain variable region locus.

19. An embryonic stem cell of claim 16 wherein the mouse lambda light chain variable region locus is replaced, in whole or in part, with a human lambda light chain variable region locus.

20. An embryonic stem cell of claim 16 wherein the heavy and light chain variable region gene loci are replaced, in whole, with their human homologs or orthologs.

21. A mouse produced from the embryonic stem cell of claim 16.

22. A mouse produced from the embryonic stem cell of claim 17.

23. A mouse produced from the embryonic stem cell of claim 18.

24. A mouse produced from the embryonic stem cell of claim 19.

25. A mouse produced from the embryonic stem cell of claim 20.

26. An antibody comprising a human variable region encoded by the genetically modified variable gene locus of claim 11.

27. An antibody of claim 26 further comprising a non-human constant region.

28. An antibody of claim 26 further comprising a human constant region.

29. A transgenic mouse having a genome comprising entirely human heavy and light chain variable region loci operably linked to entirely endogenous mouse constant region loci such that the mouse produces a serum containing an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation.

30. A transgenic mouse having a genome comprising human heavy and/or light chain variable region loci operably linked to endogenous mouse constant region loci such that the mouse produces a serum containing an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation.

31. A transgenic mouse containing an endogenous immunogobulin variable region locus that has been replaced with an homologous or orthologous human variable region locus, such mouse being produced by a method comprising:

a) obtaining one or more large cloned genomic fragments that, when combined, span the homologous or orthologous human variable region locus;

b) using bacterial homologous recombination to genetically modify the cloned genomic fragment(s) of (a) to create large targeting vector(s) for use in mouse embryonic stem cells;

c) introducing the large targeting vector(s) of (b) into mouse embryonic stem cells to replace the endogenous variable region locus in the cells;

d) using a quantitative PCR assay to detect modification of allele (MOA) in the mouse embryonic stem cells of © to identify those mouse embryonic stem cells in which the endogenous variable region locus has been replaced with the homologous or orthologous human variable region locus;

e) introducing the mouse embryonic stem cell of (d) into a blastocyst; and

f) introducing the blastocyst of (e) into a surrogate mother for gestation.

32. A transgenic mouse containing an endogenous immunoglobulin variable region locus that has been replaced with an homologous or orthologous human immunoglobulin variable region locus, such mouse being produced by a method comprising:

a) creating a LTVEC comprising a site-specific recombination site and a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the mouse immunoglobulin variable gene locus region;

b) creating a LTVEC comprising a site-specific recombination site and an upstream homology arm containing the region adjacent to the most distal mouse V gene segment, but not containing any V gene segments of the mouse immunoglobulin variable gene locus region;

c) introducing the LTVEC s of (a) and (b) into the eukaryotic cell;

d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify those eukaryotic cells in © in which the site-specific recombination sites flank the endogenous immunoglobulin variable region gene locus;

e) creating a vector containing the site-specific recombination sequences flanking all or part of the orthologous or homologous gene locus;

f) introducing the vector of (e) into the eukaryotic cells identified in step (d) such that, through recombination, the endogenous immunoglobulin variable region gene locus is replaced, in whole or in part, with the homologous or orthologous gene locus;

g) introducing the mouse embryonic stem cell of (d) into a blastocyst;; and

h) introducing the blastocyst of (e) into a surrogate mother for gestation.

33. The transgenic mouse of claims 30, 31 or 32 wherein the immunoglobulin variable region gene locus comprises one or more loci selected from the group consisting of:

a) a variable gene locus of the kappa light chain;

b) a variable gene locus of the lambda light chain; and

c) a variable gene locus of the heavy chain.

34. The method of claim 7, 8, 9, 10 or 12 wherein the mouse embryonic stem cell is derived from a transgenic mouse produced by the method of claim 31.

35. The method of claim 7, 8, 9, 10, or 12 wherein the mouse embryonic stem cell is derived from a transgenic mouse produced by the method of claim 32.

36. A method of making a human antibody comprising:

a) exposing the mouse of claim 26 to antigenic stimulation, such that the mouse produces an antibody against the antigen;

b) isolating the DNA encoding the variable regions of the heavy and light chains of the antibody;

c) operably linking the DNA encoding the variable regions of (b) to DNA encoding the human heavy and light chain constant regions in a cell capable of expressing active antibodies;

d) growing the cell under such conditions as to express the human antibody; and

e) recovering the antibody.

37. The method of claim 36 wherein the cell is a CHO cell.

38. The method of claim 36 wherein said DNA of step (b) is isolated from a hybridoma created from the spleen of the mouse exposed to antigenic stimulation in step (a).

39. The method of claim 31 wherein said DNA is isolated by PCR.

40. A method of creating, in a eukaryotic cell, an endogenous gene locus flanked downstream by a site-specific recombination site comprising:

a) creating a LTVEC comprising the site-specific recombination site, a downstream homology arm containing a region that flanks the 3′ end of the endogenous gene locus region and an upstream homology arm within the locus;

b) introducing the LTVEC of (a) into the eukaryotic cell; and

c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify those eukaryotic cells in (b) in which the endogenous gene locus is flanked downstream by the site-specific recombination site.

41. A method of creating, in a eukaryotic cell, an endogenous gene locus flanked upstream by a site-specific recombination site comprising:

a) creating a LTVEC comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5′ end of the endogenous gene locus region and a downstream homology arm within the locus;

b) introducing the LTVEC of (a) into the eukaryotic cell; and

c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify those eukaryotic cells in (b) in which the endogenous gene locus is flanked upstream by the site-specific recombination site.

42. A method of creating, in a eukaryotic cell, an endogenous gene locus flanked by site-specific recombination sites comprising:

a) creating a LTVEC comprising the site-specific recombination site, a downstream homology arm containing a region that flanks the 3′ end of the endogenous gene locus region and an upstream homology arm within the locus;

b) creating a LTVEC comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5′ end of the endogenous gene locus region and a downstream homology arm within the locus;

c) introducing the LTVEC's of (a) and (b) into the eukaryotic cell; and

d) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify those eukaryotic cells in (c) in which the site-specific recombination sites are flanking the endogenous gene locus.

43. A method of creating, in a eukaryotic cell, an endogenous immunoglobulin variable gene locus flanked by a site-specific recombination site comprising:

a) creating a LTVEC comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;

b) introducing the LTVEC of (a) into the eukaryotic cell; and

c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify those eukaryotic cells in (b) in which the site-specific recombination site flanks the downstream end of the endogenous immunovariable variable gene locus.

44. A method of creating, in a eukaryotic cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:

a) creating a LTVEC comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream homology arm within the locus;

b) introducing the LTVEC of (a) into the eukaryotic cell; and

c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify those eukaryotic cells in (c) in which the site-specific recombination sites flank the upstream end of the endogenous immunoglobulin variable region gene locus.

45. A method of creating, in a eukaryotic cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:

a) creating a LTVEC comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region, and an upstream homology arm within the locus;

b) creating a LTVEC comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream arm within the locus;

c) introducing the LTVEC s of (a) and (b) into the eukaryotic cell; and

d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify those eukaryotic cells in (c) in which the site-specific recombination sites flank the endogenous immunoglobulin variable region gene locus.

46. An endogenous immunoglobulin variable gene locus flanked by a site-specific recombination site.

47. An endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites.

48. An ES cell comprising an endogenous immunoglobulin variable gene locus flanked by a site-specific recombination site.

49. An ES cell comprising an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites.

50. A mouse produced by the cell of claim 48 or 49.